REMARKS/ARGUMENTS

Status

Claims 1-20 are under examination. Claims 31-39 are new.

Claims amended substantially as above were initially submitted in an after-final response filed November 14, 2007. In an advisory action mailed December 11, 2007, the Examiner asserted the amendments raised new issues and would not be considered. Applicants respectfully request the claims and arguments now be carefully considered and a Notice of Allowance issued. Claim 1 has been amended in part (a) to make more clear that the DNA vectors comprise a DNA segment and a vector segment, the latter being the location of the selectable and/or counter selectable markers and in part (b) to make more clear that in the practice of the claimed ligation method intermediates are produced in which in at least two DNA segments comprise exactly one such ligatable end and at least one DNA segment comprises two such ligatable ends. Referring for illustration to the cartoon shown below at page 11 of this response, the "syn1" and "syn4" DNA segments each have one ligatable end and the "syn1" and "syn4" DNA segments each have two ligatable ends.

Claim 1 is rejected as allegedly anticipated by Mandecki *et al.*

Claims 1-20 are rejected as allegedly obvious over Lebedenko *et al.* in view of both Gokhale *et al.* and Slater *et al.*

Interview

Applicants thank Examiner Popa for the courtesies shown the undersigned in a telephonic interview on March 18, 2008. The rejections of record were discussed. Applicants respectfully explained that in articulating rejections of certain claims, the Office was improperly ignoring specific elements called out in the claims.

Organization of response

The pending claims are discussed in four groups. First, independent claims 1, and dependent claims 31-34 are discussed. Second, independent claim 2, and dependent claims 3-13

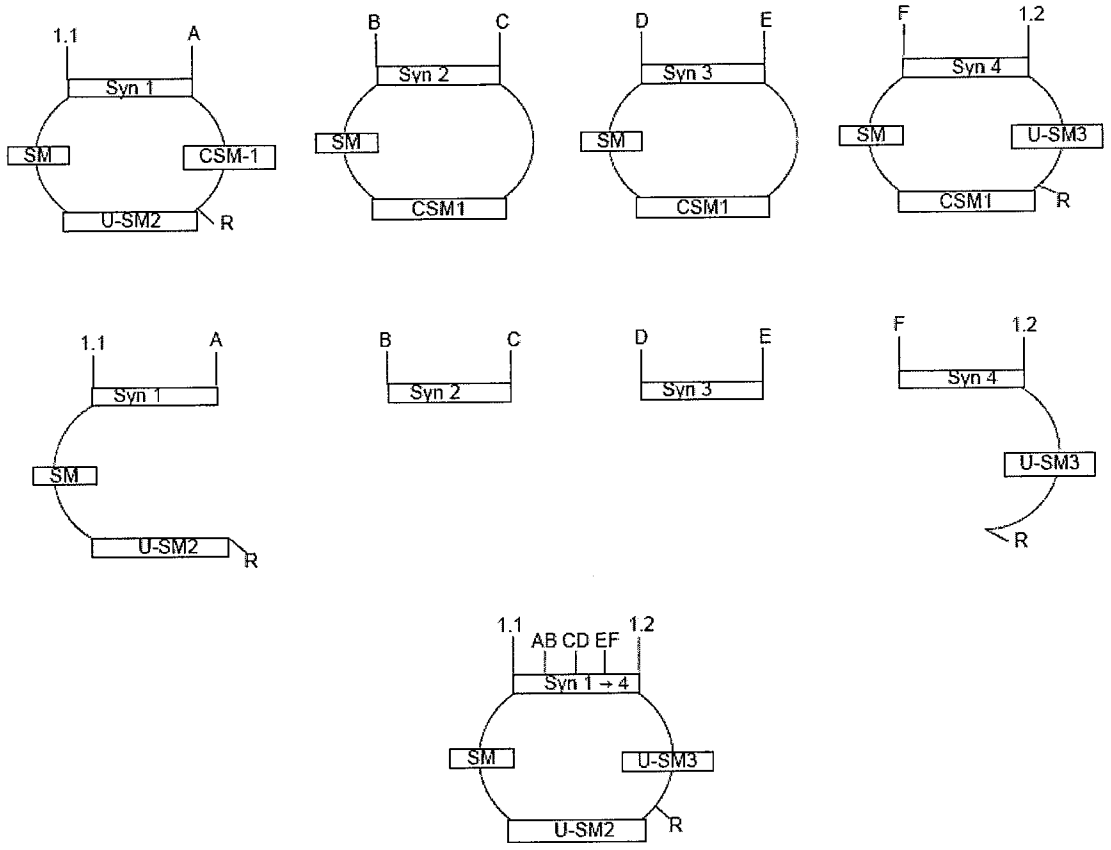
are discussed. Third, independent claim 14, and dependent claims 15-20 are discussed. Fourth independent claim 35, and dependent claims 36-39 are discussed.

The Invention

The inventors have provided a novel method that allows rapid, economic and efficient preparation of artificial genes. Unlike the prior art methods described by the Office, the invention provides a practical method for making very large synthetic genes (including genes greater than 20 kbp in length) by a process combining simultaneous ligation of multiple fragments and novel selection schemes. The claimed method makes use of a combination of three different types of vectors (see Figures 20A, B, C and D), and, in contrast to prior art methods cited by the Office, does not require multiple rounds of purification of short isolated inserts (*e.g.*, by gel electrophoresis). Eliminating the need for isolation and recloning of small polynucleotide fragments is a significant advantage that is not found in any references relied on by the Office and has which has been recognized by the scientific community.

The diagram below is based on Figure 20 of the application and provides a schematic illustration of the invention.[1] The illustration shows four vectors containing adjacent sequences or segments of the synthetic gene (labeled Syn 1-4; top row), digestion products containing the segments (middle row) and the product of simultaneous ligation of the digestion products to form a new vector comprising the four joined sequences. Keeping in mind that many other digestion products and ligation products will be formed in addition to those shown in the middle row, the method allows selection of the desired product based on a pair of selectable markers (U-SM2 and U-SM3) present in only the desired product. The method also allows elimination of undesired product based on counterselectable markers. Importantly, the resulting vector (bottom row) may be used in additional rounds of digestion, ligation and selection to create ever larger synthetic genes with a minimum of manipulation.

[1] It will be appreciated, and Applicants emphasize, that the drawing below is provided to assist the Examiner in understanding the invention, but is not intended to be limiting. One of skill reading the specification and claims would understand that numerous variations of the method are described in the specification and/or will be apparent to one of skill who has read the disclosure.



The rejections of specific groups of claims will now be addressed:

Claims 1 and 31-33

Claim 1 was rejected as allegedly anticipated by Mandecki *et al.*

Mandecki described a method in which individual segments are cloned into vectors. The inserts are excised by restriction digestion, and each isolated insert is gel-purified (see, *e.g.*, page 103, col. 2, first partial paragraph). The gel-purified fragments are then ligated to each other. The resulting ligation product is itself gel-purified. The purified ligation product is then ligated to a similarly prepared second ligation product. The full length (444 base-pair) fragment is then restriction digested, gel purified, and cloned into a vector. The necessity for isolation of fragments (*e.g.*, by gel purification) is cumbersome, slow and expensive and not practical for

synthesis of large synthetic genes. Indeed, the production of a 444-bp fragment required three different gel purification steps and numerous separate ligation steps.

Mandecki did not describe or suggest the method now claimed. Although the Office asserts that Mandecki taught that a ligation product is selected based on a selectable marker (lacZ), Applicants respectfully submit this is not correct. While Mandecki described identifying *transformants* into which inserts are cloned using lacZ, Mandecki did not describe selecting a *ligation product* based on a selectable marker. According to Mandecki's method, ligation products are selected using *gel electrophoresis* (*i.e.*, ligation products were selected on the basis of size), the very process that can be advantageously avoided using the present method.

In the Advisory Action mailed December 11, 2007, the Office asserts that in Mandecki "transformants are selected based on the presence of the final ligation product that comprises that lacZ." Applicants respectfully submit that what the Office asserts is in Mandecki is not found there. Mandecki described simultaneous ligation of gel isolated fragments (see page 103, col. 2, and page 106, last paragraph of Fig. 4, both relied on by the Office). Mandecki described that fragments 1, 2, 3, 4, 5, and 6 are isolated using PAGE, ligated together in two vector-free reaction to produce ligation produces 1-2-3 and 4-5-6 which are then purified by PAGE. The two gel purified ligation products 1-2-3 and 4-5-6 are then ligated together to produce 1-2-3-4-5-6 which is then restricted and purified by PAGE. Thus, the ligation product is selected by PAGE, not by using a selectable marker of any vector. The fragment is then cloned into a pUC plasmid and the sequence of the gene fragment confirmed by nucleotide sequencing. The Office may speculate, although it is not taught in the reference, that lacZ selection was used to distinguish pUC plasmids in which the ligation product had been inserted from empty pUC plasmids. However, even if such unsupported speculation was appropriate it would not change the situation here. The ligation product was selected using PAGE. What Mandecki may or may not have done after selecting the ligation product (sequence it, amplify it, modify it, clone it) is not relevant.

The Office also asserts that the Mandecki reference teaches using "at least three different DNA vectors". Although Mandecki described different vectors, they are not used together in a coordinated fashion, or even in the same synthesis, as are the three vector types of

the instant invention. Thus, nothing in Mandecki described a ligation method making use of three different vectors in a coordinated fashion as disclosed in the instant invention.

Moreover, claim 1 has been amended to improve clarity as discussed above. Nothing in Mandecki remotely suggested the method now claimed in which some DNA segments produced comprise a single ligatable end (the other end of the DNA segment being joined to a vector segment). Further, nothing in Mandecki suggested selection based on the presence of a selectable marker of at least one of the three DNA vectors.

Moreover, the claims that depend from claim 1 further distinguish the invention from the Mandecki reference. New Claims 31-32 specify that the selectable marker is a drug resistance gene, not described or suggested in Mandecki. Claim 34 specifies that the ligation product is selected based on two different selectable markers, each from different vector, which was not described or suggested by Mandecki.

Therefore, Applicants respectfully request this rejection be withdrawn and the claims allowed.

Claims 2-13

Claims 2-13 were rejected as allegedly obvious in view of the combination of Lebedenko *et al.*, Gokhale *et al.* and Slater *et al.*

The Office's argument is that Lebedenko *et al.* described a method for synthesis of a gene and Slater *et al.* described the use of selectable markers.[2] Initially Applicants note that almost the whole of claim 2 has not been taken into account by the Office. The Office makes the sweeping assertion that the use of selectable markers and/or counterselectable markers (apparently in any combination, in any process, and for any result) is "not innovative" because the art teaches diverse selectable and counter-selectable markers and "one of skill would know how to select markers to ensure that all inserts are present in the final product." Applicants respectfully disagree. The use of restriction sites and selectable markers to build synthetic genes

[2] Gokhale *et al.* is cited only to show motivation to make PKS genes. Applicants respectfully disagree with the Office's characterization of the reference as well as with the Office's characterization of Applicant's comments in the response filed October 17, 2006 (*e.g.*, Applicants did not argue that Gokhale *et al.* was nonanalogous art as indicated in the Office Action). In any event, Gokhale is relevant, if at all, only to claims 11 and 12 which call out polyketide synthase genes. Gokhale *et al.* does not remedy the deficiencies of the primary references (discussed below) with

as claimed and described in the specification is highly innovative and allows, *inter alia*, rapid and economical gene synthesis without the intervening isolation of fragments and required by methods such as the Lebedenko method (see Lebedenko pages 6760 col. 2, first full paragraph and page 6758, col. 1, second full paragraph).

Moreover, the Office has entirely ignored the elements of any dependent claims (other than citation of Gokhale *et al.* in connection with claims 11 and 12)[3].

Applicants believe it beyond dispute that broad unsupported statements asserting obviousness do not make a *prima facie* case. Thus, the statement "one of skill in the art *would know to use the right combination* of selectable and counter-selectable markers for the selection of the desired product" (emphasis added) is unsupported and incorrect. If this rejection is maintained, the Office is requested to be more specific about what is meant by "the right combination" and to provide some description as to *what* one of skill would supposedly know about selecting this combination, and why. Using the Lebedenko method, ligation products were selected by PAGE (see p. 6760), not using selectable markers. The Office fails to suggest any reason the Lebedenko would have been modified to add selectable and counter-selectable markers to the DNA fragments to be ligated or vectors carrying them, and does not provide any basis or indication one of skill would know *how* to accomplish what the Applicants have invented. The claimed methods were not known and are not suggested by the references relied on by the Office.

The Office asserts that one of skill would have modified the teachings of Lebedenko based on the Slater reference. Slater is directed to directional subcloning of DNA fragments but is relied on by the Office for describing use of selectable markers. With regard to Slater, in the Advisory Action mailed December 11, 2007 the Office states "it is noted the limitation of directed ligation by using DNA having a selectable and a counterselectable marker is taught by

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regard to claims 11 and 12, or any other claim. For clarity the reference is not further addressed in this analysis. Applicants do no waive any right to distinguish Gokhale reference in future prosecution.

[3] For example, the Office does not discuss how an element in Claim 3 ("selecting transformants that express said first and second selectable markers and do not express said first, second, or third counter-selectable marker") is allegedly suggested by the cited references.

[Slater]. Based on these teachings, one of skill *would know* how to use selectable and counter-selectable markers for the selection of the desired ligation product" (emphasis added).

In the Advisory Action the Office cites paragraphs 0013, 0063, 0094, 0125, and 0131 of Slater. Slater's paragraph 0013 is in the *Brief Summary* and corresponds to Slater's Figure 6A. The DNA insert A-B' is in a vector with a first selectable marker ("Drug 1") and is excised to produce the linear fragment A-B'. A second vector with a different selectable marker ("Drug 2") is digested to produce linear fragment A'-B'. The first linear fragment is ligated ("directional subcloning") to the second linear fragment to produce a vector containing both fragments (see Fig 6A). Paragraph 0013 does not in any way suggest the present invention. Slater's paragraph 0094, which is in the *Detailed Description* describes essentially the same process as described in paragraph 0013.

Slater's paragraph 0063 defines the term "selectable marker" and Slater's paragraph 0125 describes types of selectable markers.

Applicants respectfully submit that selectable markers have been known in the art for decades and those of skill have known for decades how to use such markers to select ligation products. However, nothing in Slater suggests or leads to Applicants' invention as claimed.

Accordingly, the Slater reference adds nothing to Lebedenko. Applicants respectfully reiterate the Office has not considered the invention as claimed, but appears to consider only on a few selected individual elements in isolation. For example, the Office focuses exclusively on the fact that claimed method relies, in part, on the use of selectable markers.[4] The Office repeatedly discounts the fact that Applicant's claim is directed to coordinated use of multiple different

[4] The Office is respectfully asked to revisit claim 2 and, if it is helpful, to review the cartoon on page 11 of this response. If the Applicant were claiming "A method of selecting a ligation product using selectable and counterselectable markers" the comments of the Office might (or might not) be directly relevant to the patentability of the claim. However, Applicants invention is somewhat more complicated than this and Applicant's claims are necessarily somewhat more complicated as well. Applicants agree that Slater described counter-selectable markers, as have many other prior art references. However, Slater did not suggest, alone or in combination with the other references cited by the Office, Applicant's method.

vectors, which involves markers, vector sequences, and combination of cleavage sites organized to accomplish the invention.

The Office asserts that Applicant's specification "discloses that the Type 1, 2 and 3 molecules differ only with respect to the selectable markers" (Office Action page 7, citing paragraph 0230 of the published application, US 2005/0227316). As has been noted before, this is not correct. Paragraph 0230 does not contain the word "only." Claim 2 itself plainly recites specific differing characteristics of the Type 1, Type 2 and Type 3 molecules. Finally, reference to paragraphs [0231]-[0235], original claims 21-26, the Figures (*e.g.*, Figures 20A-C), and the remainder of the specification illustrates that Type I, Type 2 and Type 3 vectors have other differences, not limited to the position of the selectable marker with relation to other vector elements. Applicants have previously noted that Claim 2 *itself* plainly recites specific differing characteristics including specified selectable markers, specified counterselectable markers, and specified cleavage sites that produce specific combinations of ligatable ends. Moreover, the claims are directed to a method: the assertions by the Office improperly ignore that the markers and cleavage sites necessarily interact in the manner specified in the claims.

Insofar as the rejection relies on Slater and Lebedenko together, Applicants again note that the Office has not explained with any specificity how or why the method of Lebedenko would have been modified to result in the present invention. Instead, the Office provides only broad assertions that "one of skill in the art *would know* how to select markers to ensure that all inserts are present in the final product" (Office Action mailed May 15, 2007, page 7, emphasis added) or "one of skill in the art *would know* to use selectable and counter-selectable markers for the selection of the desired ligation product" (Advisory Action, emphasis added). Applicants respectfully submit that, for good reason, an assertion by the Office that "one of skill would know" does not provide any legally cognizable basis for an obviousness rejection. How is a applicant to respond to such a rationale?

These assertions, in addition to being wholly unsupported, would not support a rejection for obviousness because, even if true, the references would not have suggested the present invention. That is, even if "one would know" how to modify the Lebedenko method to

incorporate selectable markers described in Slater and numerous other references (which Applicants deny), there is simply no basis for asserting that such a supposed modification would in any respect resemble Applicant's invention, in which vectors with specific characteristics are used in a specific coordinated fashion to make a synthetic gene.

To support a rejection based on obviousness, the Office must address all claim limitations, and must explain with specificity why one of ordinary skill in the art would have been motivated to carry out the claimed method. The references cited by the Office neither described nor suggested Applicant's method invention and the Office has provided no reason one of skill would have modified elements in the references to arrive at the claimed invention. Applicants respectfully request this rejection be withdrawn.

Claims 14-20

Applicants previously noted the Office has not provided any basis for the rejection of claims 14-20 and therefore has not established *prima facie* obviousness. In response (Advisory Action) the Office invites the Applicant "to carefully read the non-final Office action of 04/19/2006, describing the rejection of the embodiments recited in claims 14-20."

Applicants have reviewed the Office Action and request clarification. In that Office Action, paragraph 4 discusses claim 1. Paragraph 5 discusses claims 1-6 and 18-20. Paragraph 7 purports to discuss claims 1-20 but, in fact, on close review of paragraph 7 it remains unclear to Applicants how the Office intends to apply the Lebedenko, Gokhale and Slater references to claims 14-20. If this rejection is maintained clarification is respectfully requested.

Claims 35-39

Independent claims 35 and dependent claims 36-39 are new and have not been previously examined. These claims are directed to the method disclosed in the specification and Figures, such as the process illustrated at page 11 of this paper. Examination is respectfully requested.

Interview

Applicants again thank Examiner Popa for the courtesies shown the undersigned in the above-referenced telephonic interview, which Applicants believe was productive. Applicants respectfully request a second interview in view of the amendments and remarks above.

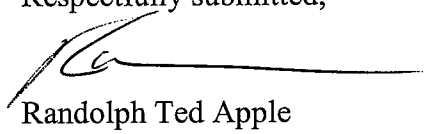
CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 650-462-5330.

Date: May 13, 2008

Respectfully submitted,



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